Optimization of a Two-Dimensional Electrophoresis Protocol for Plasma Proteomic Profiling of Obese Schizophrenia Patients

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Abstract

The proteomic approach is particularly effective for studying the association between obesity and schizophrenia. It allows for a comprehensive analysis of the complete proteome, leading to substantial breakthroughs in biomarker discovery and drug development. Isoelectric focusing (IEF) and SDS-PAGE procedures are combined in the proteomic approach known as two-dimensional electrophoresis (2-DE), which separates proteins according to their isoelectric point and mass. This study aimed to investigate optimized conditions for the 2-DE technique by focusing on the selection of an immobilized pH gradient (IPG) strip. Protein extraction was performed on pooled plasma samples from 10 obese schizophrenia patients. The extracted protein samples were loaded onto two different pH (7 cm) IPG strips. The pH ranges between (i) 3 – 10 and (ii) 4 – 7. IEF was conducted following the PROTEAN IEF Cell System protocol, followed by SDS-PAGE. The resulting gels were stained with BioSafe Coomassie stain and washed with milliQ water. The stained gels were scanned, and the images were analyzed using PD Quest software. High-abundance proteins with a molecular weight range of 60 – 80 kDa were detected on both IPG strips. The results showed that using a pH 3 – 10 IPG strip, 245 protein spots were detected and distributed throughout the gel, with a notable concentration in the middle. Whereas using a pH 4 – 7 IPG strip resulted in the detection of 321 protein spots, indicating a higher quantity of protein spots with increased intensity. This is attributed to the improved fractionation of proteins resulting from the narrower and more focused pH range. Thus, it can be inferred that utilizing this pH range will yield optimal outcomes in protein separation and analysis. This study suggests selecting a pH 4 – 7 IPG strip is the recommended choice to achieve enhanced resolution and precise detection of protein spots in plasma samples from obese schizophrenia patients when employing the 2-DE method.

INTRODUCTION

- Schizophrenia and obesity are well known among our society.
- A research in a Regional Tertiary Hospital in Malaysia concluded that the prevalence of obesity among schizophrenia patients was significantly greater than the healthy Malaysian population[1].
- The proteomic approach is one of the way to study the association of obesity and schizophrenia.
- Enabling full proteome analysis, it leads to significant breakthroughs in biomarker discovery and drug development[2].
- Proteomic method known as two-dimensional electrophoresis (2-DE) combines Isoelectric focusing (IEF) and SDS-PAGE procedures.
- In the first step, protein is separated into its charges with IEF. Whereas in the second step, the protein is separated according to its mass.

OBJECTIVE

- The main objective of this study was to explore optimal conditions for the 2-DE technique, with a specific focus on selecting an immobilized pH gradient (IPG) strip.

METHODOLOGY

1) SAMPLE PREPARATION

Protein from pooled plasma samples obtained from 10 obese schizophrenia patients, ensuring gender, race, and smoking status matching was extracted using Cleanup Kit (Bio-Rad).

2) 1ST DIMENSION OF 2-DE

The extracted protein samples were loaded onto two different pH (7 cm) IPG strips :
i) **pH 3 - 10 IPG strip** & ii) **pH 4 - 7 IPG strip**.
A passive rehydration period of 12 hours was allowed then IEF was carried out following the protocol of the PROTEAN IEF Cell System.

3) 2ND DIMENSION OF 2-DE

The IPG strips were equilibrated using an equilibration solution then SDS-PAGE was performed at a constant current of 120 V for 90 minutes.

4) GEL STAINING

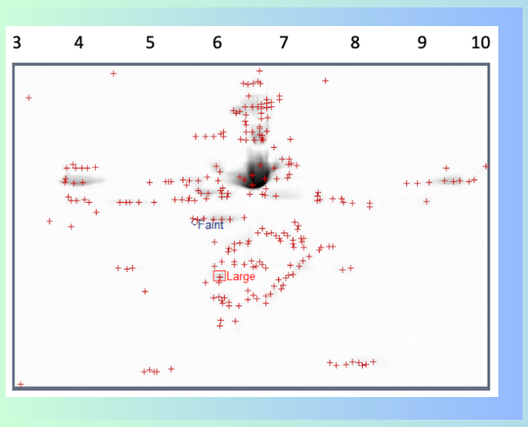
The resulting gels were subjected to 16 hour staining with BioSafe Coomassie stain, followed by washing with milliQ water.

5) IMAGING & ANALYSIS

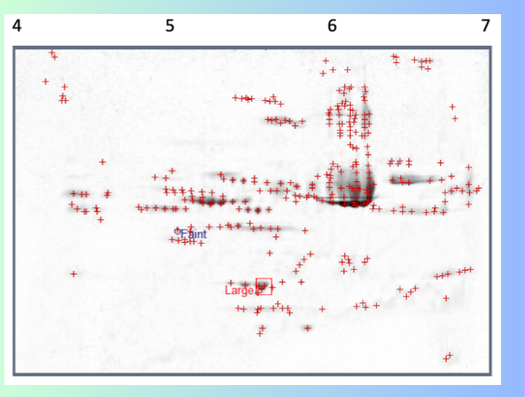
The stained gels were scanned using a GS-900 Calibrated Densitometry.
The images were analyzed using PD Quest software analysis (Bio-Rad).

RESULTS & DISCUSSION

2-DE Gel Image using a pH 3-10 IPG strip



2-DE Gel Image using a pH 4-7 IPG strip



Both IPG strips revealed the presence of high-abundance proteins, notably albumin, within the molecular weight range of 60-80 kDa.	
245 protein spots	321 protein spots
Significant accumulation of protein spots is observed in the middle	Protein spots distributed throughout the gel

- The selection of the IPG strip strongly influenced the first-dimension protein separation, based on the isoelectric charge of each protein.
- Using a narrower and more focused pH range IPG strip led to an enhanced protein fractionation[3].
- Consequently, employing a pH 4 - 7 IPG strip resulted in the detection of a higher quantity of protein spots with increased intensity.

CONCLUSION

- The utilization of a **pH 4 - 7 IPG strip** will yield the **optimal results** for protein separation and analysis compared to a pH 3 - 10 IPG strip.
- In employing the 2-DE method for plasma samples from obese schizophrenia patients, it is recommended to use a pH 4 - 7 IPG strip to achieve enhanced resolution and precise detection of protein spots.

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